CYTOLOGICAL OBSERVATIONS ON TRIPSACUM DACTYLOIDES

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Taxonomic work on *Tripsacum*[1] has indicated that *T. dactyloides* is a very complex species. It is composed of at least five different entities, one of which grows in an isolated area in western Texas and has been recognized taxonomically as *T. dactyloides* var. *occidentale* (loc. cit). Aside from this variety, *T. dactyloides* extends from central Texas and northern Kansas eastward to Connecticut and Florida (Map 1). Cytologically, *T. dactyloides* is a polyploid complex and has some races with 18 pairs of chromosomes and others with 36. The cytological work reported below extends the known range of one diploid race into Missouri and Arkansas and suggests that *T. dactyloides* may be exclusively tetraploid along the eastern seaboard.

Materials and Methods.—At the beginning of the project an earnest attempt was made to cultivate the Mexican and Guatemalan species of *Tripsacum* along with a comprehensive collection of the various forms of *T. dactyloides.* After three years of partial success this had to be abandoned as too costly in time and effort. None of these species are winter-hardy in St. Louis, even with careful attention, and even the southern Texas strains of *T. dactyloides* get gradually weaker, and a few die each winter. As a greenhouse plant *Tripsacum* does well in St. Louis during the winter, but it is difficult to bring through the long, hot summer. The collection was kept in fair condition for two years by transplanting to the breeding plot every spring and then putting the plants back into the greenhouse in September. However, when they are treated this way they do not have a regular flowering period, and some of them do not flower at all. A project of this sort could be carried out more efficiently at some such experiment station as La Jolla, California, or College Station, Texas, where the exotic species of the genus are easily grown out of doors. Since *Tripsacum* is a close relative of maize and may even be involved in the history of our cultivated varieties,[2] such a project would seem to be of fundamental importance.

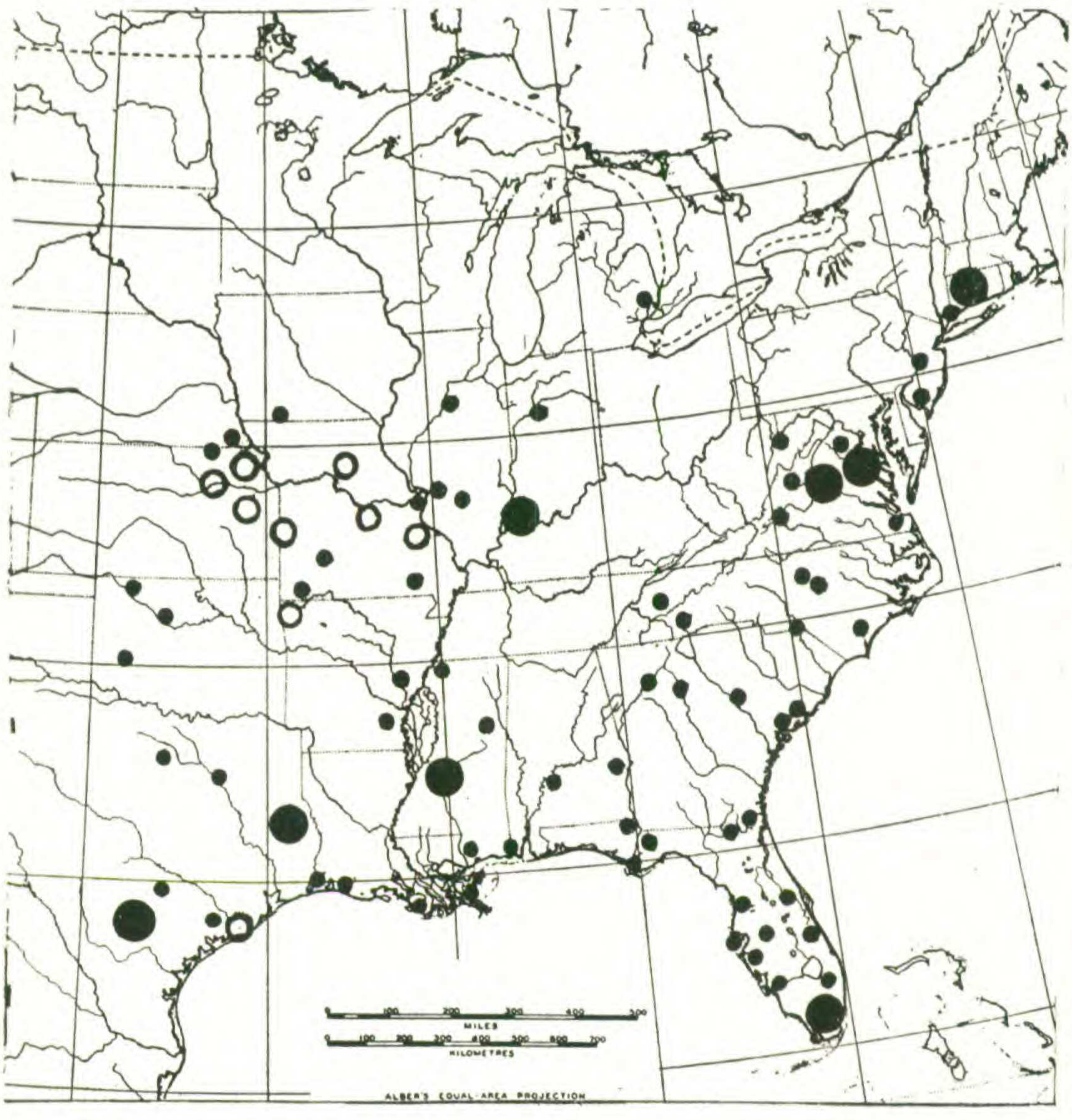
From the more northerly part of its range *Tripsacum dactyloides* was readily grown from seeds or as transplants. Seedlings grew best when planted out of doors and brought in after several weeks of cold weather. Planted out the following spring, they gave good-sized plants by the second summer. In transplanting specimens from the wild, early fall was found to be the most effective time of year and preferable to summer, late fall, or spring. The plants grew well when

[1] Cutler, Hugh C., and Edgar Anderson. A preliminary survey of the genus *Tripsacum*. Ann. Mo. Bot. Gard. 28:249-269. 1941.

[2] Mangelsdorf, Paul C., and R. G. Reeves. The origin of maize. Nat. Acad Sci., Proc. 24:303-312. 1939.

once established, and required only an occasional weeding to keep them in good condition. The following collections have been examined cytologically:

Deam	Collected by Dr. C. C. Deam 7 miles east of Lincoln City, Spencer Co., Ind.	n = 36
Dederick	Plants collected by Leslie Hubricht along stream near Dederick, Vernon Co., Mo.	n = 18
Fourche a du Clos	Plants collected 3 miles north of Bloomsdale, Ste. Genevieve Co., Mo., by Leslie Hubricht.	n = 18
Rivanna	Plants collected 3 miles north of Charlottesville, Va., and studied at the Blandy Experimental Farm of the University of Virginia.	n = 36
Rogers	Seeds collected at Rogers, Benton Co., Ark., by the Soil Conservation Service.	n = 18
Rosebud	Plants collected along roadside 13 miles n. w. of Rosebud, Gasconade Co., Mo., by Leslie Hubricht.	n = 18



Map 1. Distribution of *Tripsacum dactyloides* (exclusive of var. *occidentale*): small black dots indicate herbarium records; large black dots, 2n = 72; open circles, 2n = 36. This map summarizes the previously published information, the counts reported below, and unpublished information kindly supplied by Drs. L. R. Randolph and A. E. Longley, of the U. S. Dept. Agr.

Developing inflorescences were pickled in one part glacial acetic acid and two parts absolute alcohol, and the young anthers were smeared in Belling's aceto-carmine the next day or as soon as convenient, following McClintock's directions as to ultra-clean slides and the gentle use of heat to spread the cells. *Tripsacum* gives the appearance of going through the reduction division at a later stage in the ontogeny of the inflorescence than does maize. Secondary inflorescences pickled just after the terminal florets were clearly visible above the enclosing sheaths were found in every instance to contain pollen mother cells in all stages from early prophase to metaphase. At metaphase the PMC is so elongated that nearly all the cells lie on their sides when smeared; polar views in which the chromosomes can be counted are seldom met with. It was found more practicable to make chromosome counts in diakinesis when the chromosome pairs are showing maximum repulsion. By studying this stage it was also possible to find cells in which an approximate notion of chiasma frequencies and positions could be obtained.

Results.—Chromosome numbers have been enumerated above, with the information as to where the plants were collected. Camera-lucida drawings are shown in fig. 1. In Map 1, the distribution of these plants is shown graphically along with that of those studied by Longley[3], by Mangelsdorf and Reeves[4], and with information graciously furnished by Dr. L. R. Randolph. Taken with the morphological data reported by Cutler and Anderson, Map 1 suggests that the varieties along the eastern seaboard are amphiploid ($n = 36$) and that there is a fairly widespread race ($n = 18$) of lower ploidy along the eastern edge of the Great Plains and in the adjacent prairies. Nothing is known cytologically about the peculiar Tripsacums reported from central Illinois,[5] and the situation in Texas seems to be complex with counts of both $2n = 72$ and $2n = 36$ in the eastern part of the state. More field work in this area would be desirable but the changes wrought in the east Texas landscape by a century of intensive cultivation are so considerable that it might be very difficult, or even impossible, to determine the original relationships of these entities.

As in the related genus *Zea*, the chromosomes of *Tripsacum* undergo extreme contraction between pachytene and metaphase. Chiasma frequencies cannot be accurately determined, particularly at the later stages. At diakinesis it is often possible to make out chiasma frequency with a fair degree of objectivity, for some of the bivalents in a cell. Only very rarely are there cells in which the total frequency can be obtained. Figure 1 (above) shows a camera-lucida drawing of such a cell. The numbers given are minima; the actual total frequency might possibly be somewhat higher; it could not conceivably be any less. It gives an average frequency of just a little over two chiasmata per bivalent.

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[3] Longley, Albert E. Chromosomes in maize and maize relatives. Jour. Agr. Res. 28:673-687. 1924.

[4] *loc. cit.*

[5] Cutler and Anderson, *loc. cit.*

In the 18-paired Tripsacums no multivalent associations were recognized and there were no irregularities at metaphase. Frequently one or two bivalents would still be dividing after all the others had divided and moved to the poles, as previously described by Reeves and Mangelsdorf. When carefully examined such cells were always found to be perfectly regular in every other way and the tardy bivalents were symmetrical and were normally aligned upon the plate. It seems likely that they represent some of the larger and longer chromosomes, since it is

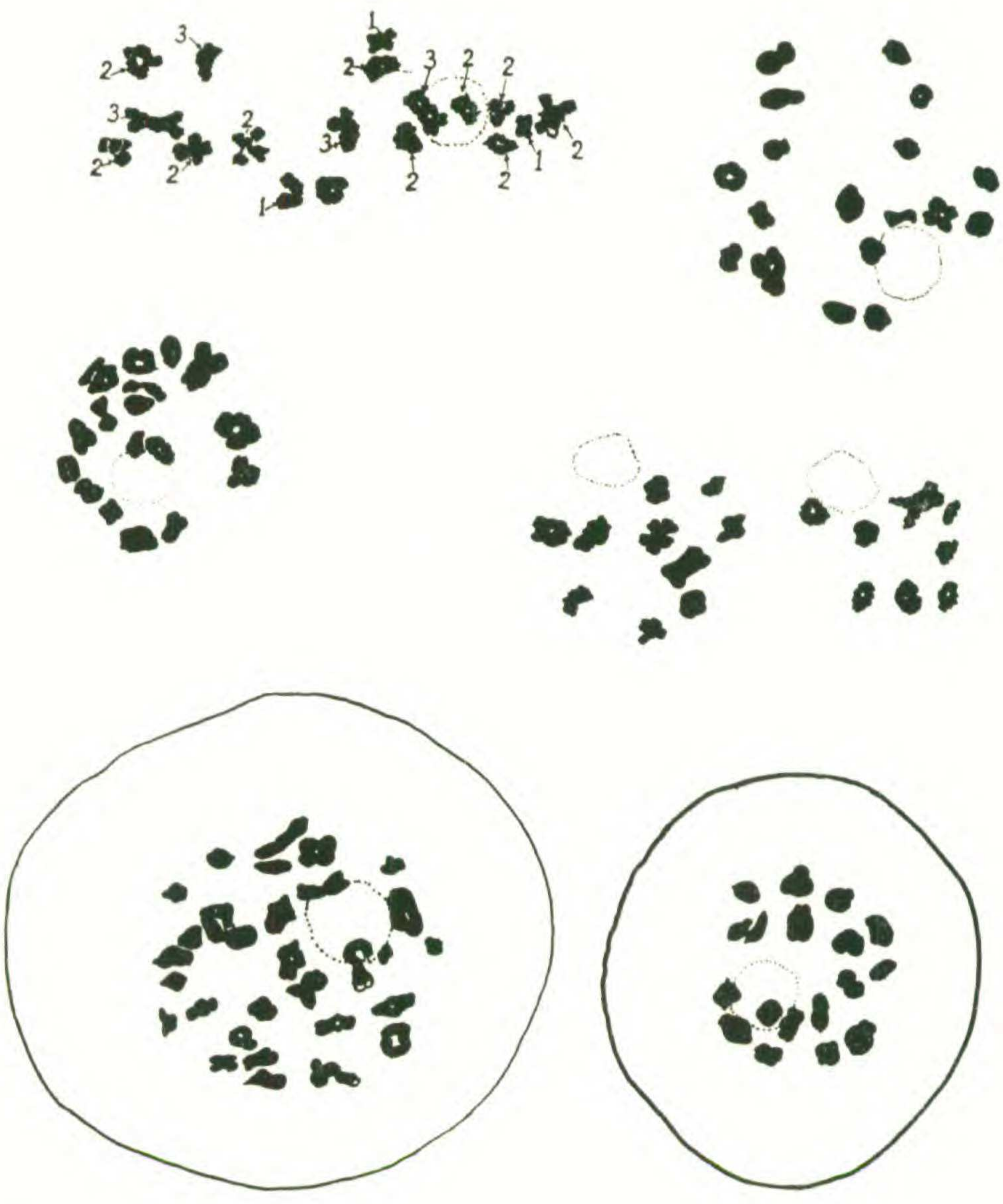

Fig. 1. Camera-lucida drawings (all to same scale) of aceto-carmine smears of *Tripsacum dactyloides* pollen mother cells at diakinesis. Nucleoli represented by light stippled outlines. All show 18 bivalents except the lower left, which has 7 quadrivalents and 28 bivalents. Upper right, plant from Rosebud, Mo.; upper left, PMC from same smear drawn at a slightly earlier stage when the chiasmata are more clearly evident, (Numbers refer to estimated numbers of chiasmata for each bivalent; configurations which would have overlapped on the drawing are drawn out separately at the left); center right, plant from Fourche a du Clos, Mo., drawn at two levels with nucleolus repeated as a reference point; center left, plant from Dederick, Mo.; lower right, plant from seed collected at Rogers, Ark.; lower left, plant from Spencer Co., Ind.

quite evident that there is a good deal of variation in size between bivalents.

In the plants with a 2n number of 72, multivalent configurations were found in every cell. Both in the material from Virginia and that from southern Indiana, cells with 30 configurations were very common. Unfortunately, the size range of bivalents is so great and the contraction can be so extreme that it is not always possible to distinguish between a small bivalent and a large univalent. Nothing was seen which, by comparison with the diploids, was certainly a univalent although there was an occasional configuration which might possibly have been one. In those multivalents whose structure could be made out with certainty only quadrivalents were recognized. These were all open rings of 4 or open chains of 4. Closed figures of 8, closed rings of 4 (all 4 united at each end) cross-shaped quadrivalents, and rings of 2 united to chains of 2 were not seen, though in such highly contracted chromosomes some of these configurations would have been difficult to make out if they had actually been present. Disjunction of the multivalents seemed to be regular at metaphase, and no micronuclei or other irregularities were noted in young microspores.

Discussion.—Eighteen is the lowest number of chromosome pairs yet reported for any *Tripsacum* (Reeves and Mangelsdorf[6]; Longley[7]), but in the closely related genus *Manisuris* there is one species with 9 pairs of chromosomes[6]. This suggests that Tripsacums with 18 pairs of chromosomes may themselves be tetraploid. Since polyploidy of a high order is very frequent in the Gramineae this does not appear at all unlikely. Furthermore, the complex morphological relationships between *Manisuris, Rottboellia, Tripsacum,* etc., suggest that they may all be part of an intricate polyploid complex. *Manisuris cylindrica,* for instance, was originally regarded as a species of *Tripsacum,* to which genus it shows a very strong natural resemblance. However, it has perfect flowers, a character which technically would remove it completely from the tribe Maydeae. By various experts it has been assigned to the genus *Rottboellia,* to *Coelorachis,* and to *Manisuris,* nor is it the only species in this group of genera whose position has been generally uncertain. The fact that *Manisuris cylindrica* is almost identical with *Tripsacum,* aside from its perfect flowers and that it has half the chromosome number of the lowest reported *Tripsacum,* would suggest that the 18-paired Tripsacums are themselves tetraploid. However, since they show little or no multivalent association, they are most probably amphidiploid derivatives of distantly related diploids. The cytological evidence would suggest that the 18-paired Tripsacums might have a cytological formula of XXYY where X and Y stand for sets of 9 chromosomes, and that *Manisuris* might be XX on the same notation. The taxonomic confusion in this group of genera is exactly what would have resulted had such inter-generic (and probably inter-tribal) reticulate relationships become established.

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[6] Reeves, R. G., and P. C. Mangelsdorf. Chromosome numbers in relatives of *Zea Mays* L. Am. Nat. **69**:633-635. 1936.

[7] *loc. cit.*

The chiasma frequencies and multivalent frequencies noted above can be used to suggest the possible relationships between the 18- and 36-paired Tripsacums. With a frequency of approximately 2 per bivalent (such as that reported above) and on the simplest possible assumptions we would expect an auto-tetraploid to form quadrivalents two-thirds of the time and pairs of bivalents one-third. (With 2 chiasmata per bivalent we expect one at each end. If among the homologous chromosomes 1, 2, 3, and 4, chromosome No. 1 pairs with No. 2, then at its other end it is equally likely that it pair with 2, 3, or 4. Pairing with 3 or 4 will produce quadrivalents, with 2 will produce 2 bivalents.) If the 36 Tripsacums are auto-polyploids we would expect two-thirds of them to produce quadrivalents and one-third bivalents. This would result in 12 quadrivalents and 12 bivalents, a total of 24 configurations per cell. If, however, they are amphipolyploids between related tetraploids (some such relationship as that between XXYY and XXZZ) then we would expect 30 configurations, the number actually observed. (Using XXYY and XXZZ to diagram the putative parental Tripsacums then we would expect the 9 sets of X's to form 6 quadrivalents and 6 bivalents, the Y's to form 9 bivalents and the Z's to form 9 bivalents, a total of 30 configurations).

The above computations should not be taken too seriously since they probably represent an over-simplification of what occurs and since the chiasma frequencies are based on estimates. However, they are put forward with more confidence since the observations preceded the computations. The estimate of 30 configurations was made so repeatedly in different smears of the 36-chromosomed plants from Indiana that a search was made through the records to find what has been noted about the Virginia plant when it was studied two years before. When the notes showed that it too had an overwhelming number of counts of 30, the computations were undertaken to see on what conditions such a number could be obtained. Since the Virginia and Indiana plants show certain constant morphological differences from the 18-paired plants of the prairies and Great Plains the simplest hypothesis was to assume that if the 18's were tetraploids of the constitution XXYY then the 36's were octoploids of the constitutionXXYYXXZZ. The fact that the computations agreed exactly with the observations is more likely a happy coincidence than it is an exact scientific verification. It does, however, seem fairly certain that the 36's are not autopolyploids and that they must have roughly some such formula as the one suggested.

SUMMARY

1. The difficulties of maintaining a collection of *Tripsacum* plants are described.

2. Chromosome counts are reported for *Tripsacum dactyloides* collected at various points throughout its range. In the prairies and Great Plains this species has 18 pairs of chromosomes. Along the eastern seaboard it has 36 and in Texas both numbers have been reported.

3. The 18-paired plants undergo regular meiosis with no multivalent associations. Those with 72 chromosomes averaged 6 quadrivalents and 24 bivalents. Chiasma frequency in the former is at least 2 per bivalent.

4. On the basis of these observations and the general morphology of *T. dactyloides,* it is suggested that the 18-paired varieties of *Tripsacum* may be amphidiploids of the formula XXYY and that the 36-paired varieties arose as octoploids (XXYYXXZZ) between XXYY and XXZZ varieties.